

# REPORT ABOUT THE WORK CARRIED OUT ON SWORDFISH (*Xiphias gladius*) ALONG ITS MEDITERRANEAN DISTRIBUTION USING PROTEIN ELECTROPHORESIS

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## SUMMARY

This work presents the preliminary results of the work being carried out to learn about the genetic structure of the Mediterranean distribution of swordfish (*Xiphias gladius*). This work is part of the European Project EEC/XIV-1/MED/91-012 on "Characterization of large pelagic stocks in the Mediterranean".

## RESUME

On trouvera dans ce document les premiers résultats de notre travail sur la structure génétique de la distribution méditerranéenne de l'espadon (*Xiphias gladius*). Ce travail est réalisé dans le cadre du Projet CEE/XIV-1/MED/91-012 intitulé "Characterization of large pelagic stocks in the Mediterranean".

## RESUMEN

Se presentan los primeros resultados acerca del trabajo que se realiza sobre la distribución mediterránea del pez espada (*Xiphias gladius*), a fin de conocer su estructura genética y poder inferir la posible estructuración de la población. Este trabajo forma parte del Proyecto CEE/XIV-1/MED/91-012 sobre "Characterization of large pelagic stocks in the Mediterranean".

## Introduction

An important component of fishery management is knowledge of the genetic population structure of the species in question. In the absence of such information, a particular fishery is at risk of being improperly regulated. If a species is genetically subdivided but is managed for maximum sustained yield as a panmictic unit, the weaker populations suffer over-harvest and the stronger ones are under-harvested. These problems persist even if a genetic structuring is assumed to exist but is inadequately known (Allendorf *et al.*, 1987).

Previous studies on this field have been carried out in different pelagic species. About swordfish, several morphometric studies have been carried out on its west and east distribution (Beckett, 1970; Radtke and Hurley, 1983; De Metrio and Megalofonou, 1987; De Metrio *et al.*, 1988), and its suggest that it may exist some subpopulations structure on swordfish. Concerning genetic information, we only know the unpublished data of Johnson (1987) on protein electrophoresis, and the studies of Alvarado (1991) on Atlantic swordfish and of Zouros *et al.* (1991) on Mediterranean swordfish, both using mtDNA analysis.

This paper present the preliminary work carried out to examine the genetic variation of the swordfish (*Xiphias gladius*), along its Mediterranean distribution, caught in waters under Spain, French, Italian and Greece jurisdiction. Fish were captured by trawl in geographically diverse areas within the proposed study area and are being analyzed by protein electrophoresis in order to know the genetic structure of each sampled area.

## Materials and methods and Results

We are sampled several areas along the Mediterranean Sea: Alborán Sea, in Spain; Ligurian and Adriatic Seas in Italy and Aegean Sea in Greece, during the 1993 and 1994 fishing seasons. For each area, 50 individuals were caught to be used in this study. Just now, we are done the analysis of the swordfish samples of the 93 season and we will start the analysis of the samples of this year that we just are receiving. The analysis are being carried out by protein electrophoresis on different tissues: liver, heart and muscle.

Unprocessed fish were immediately frozen and maintained at low temperature (- 30°C) until its transportation to our laboratory, where it is kept at - 80°C prior to electrophoretic analysis. Tissue extraction, electrophoresis and procedures for visualizing proteins generally followed the methods outlined in Aebersold *et al.* (1987). Extracts from tissues including liver (L), heart (H) and skeletal muscle (M) were electrophoretically screened for resolution and activity with buffer systems.

From 26 different enzymatic systems that have been studied, only 23 showed a good activity to be a reflection of 36 genetic loci. Seventeen of these loci have showed polymorphism in some of the 4 sampled locations, although only eleven of these polymorphic loci have showed a good resolution with an interpretable genetic pattern to be used in the study. A summary of this data is represented on Tables 1 and 2. Table 1 show the enzym systems analyzed and its nomenclature; Table 2 show the enzym systems that produced distinct banding patterns, loci reported and best tissue and buffer used for each locus. Genetic interpretations of these patterns followed principles outlined in Utter *et al.* (1987). Genetic nomenclature followed the suggestions of Shaklee *et al.* (1990).

Allelic and genotypic data will be analyzed by the Biosys Program (Swofford and Selander, 1981). For each locus in the different populations, deviations from Hardy-Weinberg proportions will be tested using the independence chi-square test and exact probabilities test. Allele frequency differences among samples were tested by contingency chi-square analysis. With these data, standard genetic distance values will be calculated according to Nei (1972) and a dendrogram will be constructed using the unweighted pair-group method with arithmetic average (UPGMA; Sneath and Sokal, 1973).

### Future directions

Requirements for further investigation should include a broad temporal and geographic sampling of the swordfish in the spawning areas and along the main areas of its west and east distribution followed by an extensive genetic and morph-meristic analysis of these samples. Technologies involving analyses of mitochondrial and nuclear DNA should be applied within areas appearing to be genetically homogeneous on the basis of extensive surveys of electrophoretically detected variants of protein-coding loci. Following the collection and analyses of such full sets of genetic data, a firmer understanding of the population structure of this valuable resource will provide a sounder biological basis for its management. In this sense, we have planned to continue this project by sequencing different regions of mtDNA like control region of D-loop or cytochrom b and c.

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Table 1. Enzyme systems analyzed, coding commission and nomenclature.

AAT	2.6.1.1.	Aspartate aminotransferase
ACP	3.1.3.2.	Acid phosphatase
ADH	1.1.1.1	Alcohol dehydrogenase
AH	4.2.1.3	Aconitate hydratase
CAT	1.11.1.6.	Catalase
EST	3.1.1.1.	Esterase
FUM	4.2.1.2.	Fumarate hydratase
G3PDH	1.1.1.8.	Glycerol-3-phosphate dehydrogenase
GAPDH	1.2.1.12.	Glyceraldehyde-3-phosphate dehydrogenase
GDA	3.5.4.3.	Guanine deaminase
GDH	1.1.1.47.	Glucose dehydrogenase
GLUDH	1.4.1.3	Glutamate dehydrogenase
GPI	5.3.1.9.	Glucose-6-phosphate isomerase
IDH	1.1.1.42.	Isocitrate dehydrogenase
LDH	1.1.1.27.	Lactate dehydrogenase
LGL	4.4.1.5.	Lactoyl glutathione lyase
MDH	1.1.1.37.	Malate dehydrogenase
ME	1.1.1.39.	Malic enzyme (NAD <sup>+</sup> )
MEL	1.1.1.40	Malic enzyme (NADP <sup>+</sup> )
MPI	5.3.1.8.	Mannose-6-phosphate isomerase
PEP	3.4.13.-	Peptidase
PGDH	1.1.1.44.	Phosphogluconate dehydrogenase
PGM	5.4.2.2.	Phosphoglucomutase
PK	2.7.1.40.	Pyruvate Kinase
SOD	1.15.1.1.	Superoxide dismutase
XDH	1.2.3.27.	Xantine dehydrogenase

Table 2. Enzymatic locus abbreviations, best tissue and buffer conditions on *X.gladus*.

Buffers: 1 AC, 2 TC/LB, 3 TBE, 4 POULIK, 5 TC, 6 TBE+NAD, 7 TP  
 Tissue: F liver, C heart, M muscle.  
 m.r. bad resolution  
 M: monomorphic; P: polymorphic

LOCUS	NOM.	BUFFER	TISSUE	ALLELE
AAT-1	2.6.1.1.	1, 2	F, M	M
AAT-2				M
ACP	3.1.3.2.	3, 4	all	M
ADH	1.1.1.1.	1	F	m.r.
AH	4.2.1.3.	1	F	m.r.
CAT	1.11.1.6.	2, 4	F	P (70/100)
EST-1	3.1.1.1.	2	C, F	M
FUM	4.2.1.2.	4	C	M
G3PDH-2	1.1.1.8	1	F	M
G3PDH-1				?
GAPDH-1	1.2.1.12	6, 1, 2	C	P (-100/-200)
GAPDH-2				P (100/115)
GDA	3.5.4.3.	7, 1	F	M
GDH	1.1.1.47.	1, 4	F	P (90/100)
GLUDH	1.4.1.3.	1, 4	F, C	P?
GPI-1	5.3.1.9.	1, 2	C, F, M	M
GPI-2				M
GPI-3				P?
IDH-1	1.1.1.42.	1	C,F	P?
LDH-1	1.1.1.27.	1, 4	M	P?
LDH-2			C	P?
LGL	4.4.1.5.	1, 2, 3	all	M
MDH-1	1.1.1.37.	1	C	P (100/150)
MDH-2			C	P (100/130)
ME-1	1.1.1.39.	2, 3	C	P (90/100)
ME-2			C	P (100/105)
ME-3			F	P (90/100/110)
MEL	1.1.1.40.	1, 4	C	M
MPI	5.3.1.8.	7	C	M
PEP-LG	3.4.13.-	2	C	P (100/120)
PEP-LGG	3.4.13.-	2	C	M
PEP-PAP	3.4.13.-	2	C	M
PGDH	1.1.1.44.	5, 3	C	P (90/100)
PGM	5.4.2.2.	1, 4	C,F	P?
PK-1	2.7.1.40	1	C	M
PK-2				M
SOD-1	1.15.1.1.	1	F, C	M
SOD-2				M
XDH	1.2.3.27	2, 3	F	M